

Performance of Rapid Antigen Tests and Inflammatory Markers in SARS-CoV-2: Lessons for Future Respiratory Pandemics

Ata Ul Mustafa¹, Muhammad Hidayat Rasool^{1*}, Bilal Aslam¹, Muhammad Shafique¹

ABSTRACT

OBJECTIVE: To evaluate the diagnostic performance of rapid antigen tests (RATs) and the utility of inflammatory biomarkers in symptomatic and asymptomatic SARS-CoV-2 cases among healthcare workers and the general population in Punjab, Pakistan.

METHODOLOGY: This cross-sectional study enrolled 1,500 participants (734 symptomatic; 766 asymptomatic), including healthcare workers (HCWs) and the general population (GP), across three cities in Punjab, Pakistan, between November 2021 and July 2022 at the Institute of Microbiology, Government College University, Faisalabad, Pakistan. Participants underwent parallel RT-PCR and Rapid antigen testing (RAT) from nasopharyngeal swabs. Serum/plasma biomarkers (LDH, D-dimer, CRP, IL-6) were quantified using standardized assays.

RESULTS: Symptomatic individuals showed significantly higher SARS-CoV-2 positivity than asymptomatic individuals by PCR (92.3% vs. 38.8%), RAT (89.8% vs. 4.0%), and IgG serology (82.2% vs. 59.7%) (all $p < 0.0001$). Symptomatic cases exhibited markedly elevated biomarkers (e.g., LDH: 394 U/L vs. 195 U/L; CRP: 29 mg/L vs. 0.81 mg/L; all $p < 0.001$). HCWs had higher PCR positivity (68.0% vs. 62.6%; $p = 0.041$) and higher levels of LDH, CRP, and IL-6 ($p < 0.001$) than GP. RAT sensitivity was high in symptomatic cases (97.3%) but low in asymptomatic individuals (10.2%).

CONCLUSION: Rapid antigen tests excel at detecting symptomatic SARS-CoV-2 infections but are unreliable for asymptomatic infections. Biomarkers robustly indicate systemic inflammation and clinical risk in symptomatic individuals and HCWs. Context-based strategies are essential. For example, using RATs to control the spread among people with symptoms while using biomarkers to assess disease severity in high-risk groups, especially in low-resource settings, can improve the pandemic response.

KEYWORDS: SARS-CoV-2, Inflammatory biomarkers, Healthcare workers, Diagnostic performance, Resource-limited settings, Pandemic preparedness.

INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, began in late 2019. It quickly spread into a global pandemic, overwhelming healthcare systems and resulting in millions of deaths¹. Rapid recognition of infected individuals, especially during presymptomatic or early symptomatic periods when viral loads are highest, reduces secondary transmission and disease severity by enabling timely isolation and interventions^{1,2}.

RATs offer significant pandemic-control benefits over RT-PCR, including point-of-care use, faster turnaround, lower cost, and the ability to support high-frequency testing with same-day isolation³. However, RATs are less sensitive (68–99%) than RT-PCR, particularly during reduced viral loads or during symptomatic or asymptomatic infection^{4,5}. Thus, RATs excel as public health tools for curbing transmission, whereas PCR remains the diagnostic gold standard for individual diagnosis when sensitivity is the primary concern⁴. Inflammatory biomarkers are critical for predicting COVID-19 severity and addressing RAT

limitations. Markers such as lactate dehydrogenase (LDH), D-dimer, C-reactive protein (CRP), and interleukin-6 (IL-6) rise days before clinically evident symptoms, signalling hyperinflammation (e.g., a cytokine storm) and increased risk of organ damage^{6,7}.

The diagnostic accuracy for SARS-CoV-2 tests is not universally generalizable, particularly in genetically and epidemiologically diverse populations. In Pakistan, there is a lack of integrated data on the combined evaluation of RAT performance and inflammatory biomarkers (LDH, CRP, D-dimer, IL-6) in both healthcare workers and the general population. This evidence gap limits the development of diagnostic and prognostic strategies tailored to local needs and may result in the misapplication of international data in future respiratory pandemics. Therefore, this study was undertaken to: (i) assess the diagnostic performance of RATs compared with RT-PCR in symptomatic and asymptomatic individuals; and (ii) evaluate the levels of key inflammatory biomarkers among SARS-CoV-2 cases, with subgroup analyses for healthcare workers and the general population.

The findings aim to generate context-specific evidence to guide pandemic response strategies in resource-limited settings.

¹Institute of Microbiology, Government College University Faisalabad, Faisalabad, Pakistan

Correspondence: drmhrasool@gcuf.edu.pk

doi: 10.22442/jlumhs.2026.01370

Received: 08-08-2025

Revised: 25-11-2025

Accepted: 28-11-2025

Published Online: 03-02-2026



METHODOLOGY

A cross-sectional molecular epidemiological study was conducted across three major cities in Punjab, Pakistan (Lahore, Faisalabad, Sargodha) between November 2021 and July 2022. The study enrolled 1,500 participants presenting with suspected SARS-CoV-2 infection, comprising 734 symptomatic and 766 asymptomatic individuals. The convenience sampling was used, and participant recruitment was conducted entirely in person. No online or social media-based survey methods were used. The sample distribution was as follows: Lahore contributed 471 participants (31.4%; 249 symptomatic, 222 asymptomatic), Faisalabad 538 (35.9%; 261 symptomatic, 277 asymptomatic), and Sargodha 491 (32.7%; 256 symptomatic, 235 asymptomatic) (**Figure 1**). The sample size was determined using the Raosoft sample size calculator (Raosoft, Inc., USA), and all procedures adhered to the Punjab Health Care Commission guidelines⁸.

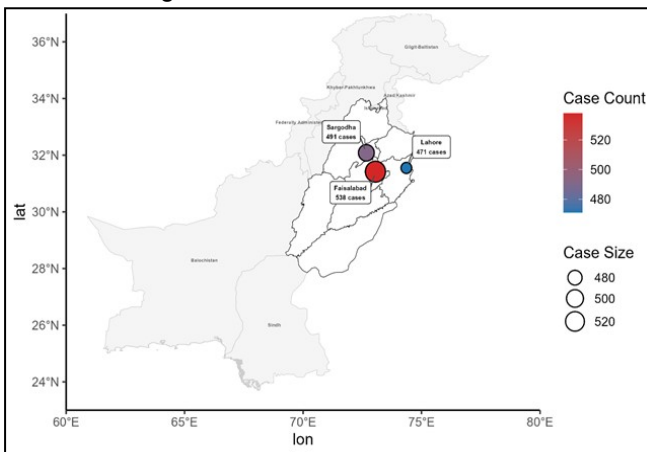


Figure 1: Geographic Distribution of Study Participants Across Three Cities in Punjab, Pakistan

The map illustrates the number of enrolled participants from three major cities (Faisalabad, Sargodha, and Lahore). The relative participant counts are indicated by proportional markers and color intensity reflecting the distribution of the study population across the region.

Data and Sample Collection

Demographic, clinical, and occupational data, including age, gender, symptom status, vaccination history, profession, residence, and travel history, were recorded using standardized questionnaires. Paired biological samples were collected from each participant: nasopharyngeal swabs preserved in viral transport medium (VTM) for viral detection, and venous blood drawn into 3.2% Sodium Citrate tubes (for plasma) for D-Dimer measurement and serum separator tubes (for serum) for serological and biomarker analysis. Sample temperatures were maintained between 2°C and 8°C throughout transport from collection sites to the laboratory using insulated

cold boxes. Cold chain integrity was monitored using an HTC-2 digital thermometer to track temperature and humidity, ensuring sample stability for molecular detection. Upon arrival, samples were stored at 2–8°C in the laboratory before processing.

SARS-CoV-2 Detection

Nasopharyngeal swabs underwent parallel batch-wise testing via RT-PCR and rapid antigen test (RAT) upon receipt. For RT-PCR analysis, RNA extraction was performed using the HERO 32 Magnetic Bead System (Ascend Biotechnology, China). Amplification was followed using a TaqMan-based assay (ACON Biotech, China) targeting the N, E, and ORF1ab genes on an Applied Biosystems QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, USA). The thermal cycling protocol comprised reverse transcription at 50°C for 20 minutes, followed by 45 cycles of denaturation at 94°C for 15 seconds and annealing/extension at 60°C for 30 seconds. Samples with cycle threshold (Ct) values < 36 were considered positive per manufacturer guidelines.

Concurrent RAT testing utilized the Abbott Panbio™ COVID-19 Ag Rapid Test Device, with results interpreted visually 15–20 minutes after sample application.

Serological and Biomarker Analysis

Serum samples were tested for SARS-CoV-2 IgG antibodies using the Abbott Panbio COVID-19 IgG Rapid Test Device, with results interpreted visually at 10–20 minutes. Biomarker quantification included: serum lactate dehydrogenase (LDH) measured via colorimetric enzymatic assay on a Roche Cobas c501 analyzer (reference range: 135–214 U/L); plasma D-dimer quantified by immunoturbidimetric assay on (Roche Cobas c501; reference range: 0–0.5 µg/mL); serum C-reactive protein (CRP) assessed via turbidimetric immunoassay (Roche Cobas c501; reference: <10 mg/L); and serum interleukin-6 (IL-6) measured using high-sensitivity ELISA (Monobind Inc., USA) with absorbance read at 450 nm (reference: <35 pg/mL).

Statistical Analysis

Data analysis utilized R (v4.4.1; RStudio 2023.12.1), Python (v3.13.5), and IBM SPSS Statistics (v23). Analytical approaches included chi-square/Fisher's exact tests for prevalence comparisons, Mann-Whitney U/Kruskal-Wallis tests for biomarker level comparisons, Spearman's rank correlation for association analyses, and multivariate regression to adjust for confounding variables.

RESULTS

Demographic Correlates of Symptomatic SARS-CoV-2 Infection

Descriptive analysis of participant characteristics revealed a significant association between gender and symptomatic status ($p < 0.0001$), with a higher proportion of symptomatic individuals being male (63.8%) compared to asymptomatic individuals (45.9%). However, no statistically significant

differences were observed between the two groups with respect to age (mean: 34.1 ± 9.7 vs. 34.4 ± 10.0 years; $p = 0.507$), occupation, residence (urban vs. rural), or COVID-19 vaccination status (**Table I**). The age distribution differed slightly by symptom status, with symptomatic individuals generally older than asymptomatic participants, as shown in **Figure 2**.

Table I: Demographic Characteristics of patients

Variable	Symptomatic (n=766)	Asymptomatic (n=734)	p-value (test)
Age (years)	34.1 ± 9.7	34.4 ± 10.0	0.507 (Independent t-test)
Gender			
Female	277 (36.2%)	397 (54.1%)	<0.0001 (X^2)
Male	489 (63.8%)	337 (45.9%)	<0.0001 (X^2)
Occupation			
Essential Worker	184 (24.0%)	160 (21.8%)	0.336 (X^2)
Frontline Worker	115 (15.0%)	110 (15.0%)	1.000 (X^2)
Healthcare Worker	213 (27.8%)	202 (27.5%)	0.947 (X^2)
Housewife	75 (9.8%)	70 (9.5%)	0.937 (X^2)
Other	18 (2.3%)	19 (2.6%)	0.895 (X^2)
Shopkeeper	62 (8.1%)	70 (9.5%)	0.371 (X^2)
Student	82 (10.7%)	88 (12.0%)	0.482 (X^2)
Unemployed	17 (2.2%)	15 (2.0%)	0.955 (X^2)
Residence			
Urban Residence	463 (60.4%)	449 (61.2%)	0.814 (X^2)
Rural Residence	303 (39.6%)	285 (38.8%)	0.814 (X^2)
Vaccination status			
Vaccinated	258 (33.7%)	240 (32.7%)	0.727 (X^2)
Unvaccinated	508 (66.3%)	494 (67.3%)	0.727 (X^2)

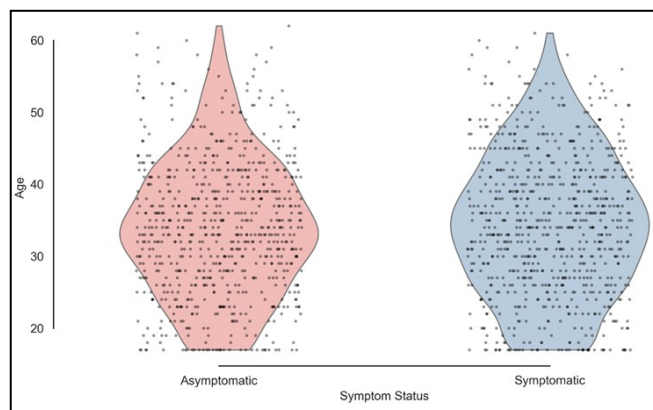


Figure 2: Age Distribution by Symptom Status Among COVID-19 Participants

The violin plot depicts the distribution of age among asymptomatic and symptomatic individuals. Each violin shape illustrates the probability density of the age distribution at different values. The width of the violin represents the relative frequency of participants at a given age.

Diagnostic Test Positivity Among Symptomatic vs. Asymptomatic Individuals

A comparison of SARS-CoV-2 diagnostic test results between symptomatic and asymptomatic individuals revealed significant differences across all testing modalities (**Table II**). Among the 1500 participants, 66.1% tested positive by PCR, with a notably higher positivity rate in symptomatic individuals (92.3%) than in asymptomatic ones (38.8%), a statistically significant difference ($X^2 = 476.08$, $p < 0.0001$). Similarly, rapid antigen test (RAT) positivity was observed in 47.8% overall, with 89.8% in the symptomatic group and only 4.0% in the asymptomatic individuals ($X^2 = 1104.16$, $p < 0.0001$). The presence of IgG antibodies was also significantly more frequent in symptomatic participants (82.2%) than in asymptomatic ones (59.7%) ($X^2 = 92.04$, $p < 0.0001$).

Table II: Diagnostic Test Results by Symptom Status among Randomly Sampled Individuals Screened for COVID-19

Test Result	Overall (n = 1500)	Symptomatic (n = 766)	Asymptomatic (n = 734)	Statistical Test Chi-square, p-value)
PCR Positive	992 (66.1%)	707 (92.3%)	285 (38.8%)	$X^2 = 476.08$, $p = 0.0000$
PCR Negative	508 (33.9%)	59 (7.7%)	449 (61.2%)	
RAT Positive	717 (47.8%)	688 (89.8%)	29 (4.0%)	$X^2 = 1104.16$, $p = 0.0000$
RAT Negative	783 (52.2%)	78 (10.2%)	705 (96.0%)	
IgG Positive	1068 (71.2%)	630 (82.2%)	438 (59.7%)	$X^2 = 92.04$, $p = 0.0000$
IgG Negative	432 (28.8%)	136 (17.8%)	296 (40.3%)	

Inflammatory and Coagulation Biomarker Profiles in SARS-CoV-2 Cases

A comparative analysis of inflammatory and coagulation biomarkers between symptomatic and asymptomatic individuals revealed statistically significant differences in all measured parameters ($p < 0.001$ for each) as shown in **Table III**. Median serum levels of LDH, CRP, D-dimer, and IL-6 were markedly elevated in symptomatic patients compared to their asymptomatic counterparts. Specifically, LDH levels were nearly twice as high in symptomatic individuals (median: 394 U/L vs. 195 U/L), while CRP levels showed an even greater disparity (29 mg/L vs. 0.81 mg/L). Similarly, symptomatic individuals exhibited significantly elevated D-dimer (1.3 $\mu\text{g/mL}$ vs. 0.3 $\mu\text{g/mL}$) and IL-6 levels (50 pg/mL vs. 5 pg/mL). The distribution of inflammatory markers (CRP, D-dimer, IL-6) stratified by symptom status among COVID-19 patients is shown in **Figure 3**. Chi-square test results confirm that differences in biomarker elevations between groups are statistically significant, suggesting a strong association between symptom manifestation and systemic inflammatory response (**Table IV**).

Table III: Comparison of Biomarker Levels in Symptomatic and Asymptomatic Individuals

Biomarker	Group	Median	IQR	Min–Max	p-value (Mann–Whitney U)
LDH (U/L)	Symptomatic	394	56.0	149 – 547	< 0.001
	Asymptomatic	195	25.6	135 – 268	
CRP (mg/L)	Symptomatic	29	13.8	1.3 – 59.3	< 0.001
	Asymptomatic	0.81	0.2	0.42–17.6	
D-Dimer (µg/mL)	Symptomatic	1.3	0.6	0.0 – 3.1	< 0.001
	Asymptomatic	0.3	0.2	0.0 – 0.6	
IL-6 (pg/mL)	Symptomatic	50	14.3	19 – 80.2	< 0.001
	Asymptomatic	5	1.0	2.5 – 72	

Table IV: Frequency and Percentage of Elevated Biomarkers Among Symptomatic and Asymptomatic Individuals

Biomarker	Threshold	Symptomatic (n=766)	Asymptomatic (n=734)	p-value (X ² test)
LDH Elevated	> 214 U/L	741 (96.7%)	94 (12.8%)	< 0.001
D-Dimer Elevated	> 0.5 µg/mL	692 (90.3%)	45 (6.1%)	< 0.001
CRP Elevated	> 10 mg/L	686 (89.6%)	31 (4.2%)	< 0.001
IL-6 Elevated	> 35 pg/mL	710 (92.7%)	32 (4.4%)	< 0.001

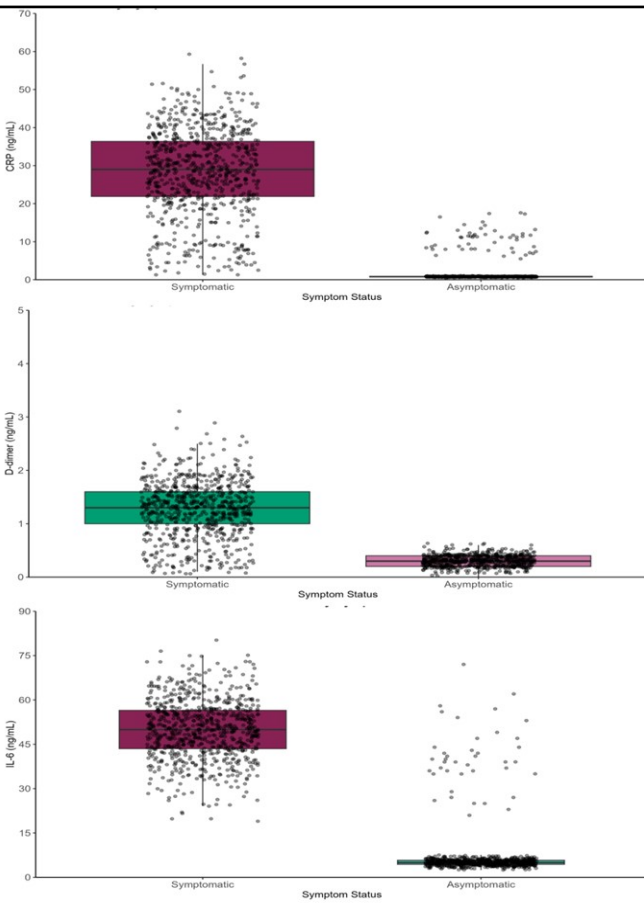


Figure 3: Inflammatory Laboratory Markers Stratified by Symptom Status in COVID-19 Patients

Boxplots show the distributions of CRP, D-dimer, and IL-6 levels in asymptomatic and symptomatic individuals. Individual data points are overlaid on boxplots to illustrate variability and density within groups. Median values are indicated by horizontal bars within each box. Outliers and wider interquartile ranges are more prominent among symptomatic individuals, suggesting greater heterogeneity in inflammatory response.

Correlation analysis was conducted to assess the relationships between inflammatory and coagulation markers (D-dimer, CRP, IL-6) and patient age in COVID-19 cases. As shown in both Pearson and Spearman correlation heatmaps (**Figure 4**), a strong positive correlation was observed among the three biomarkers. The highest correlation was noted between IL-6 and CRP (Pearson: $r = 0.78$; Spearman: $r = 0.73$), followed by IL-6 and D-dimer (Pearson: $r = 0.74$; Spearman: $r = 0.67$). In contrast, age demonstrated a very weak correlation with all laboratory parameters (Pearson r range: 0.05–0.11; Spearman r range: 0.04–0.09).

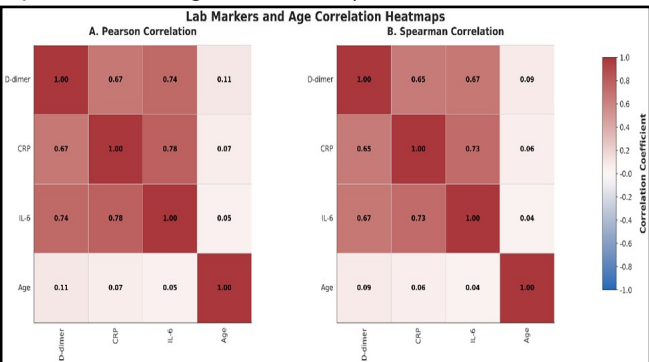


Figure 4: Correlation heatmaps of laboratory markers and age in COVID-19 patients

(A) Pearson correlation and (B) Spearman correlation matrices illustrate the relationships among D-dimer, CRP, IL-6, and age. Each cell displays the corresponding correlation coefficient, with a color gradient ranging from light (negative correlation) to dark (positive correlation). Strong positive correlations were observed among D-dimer, CRP, and IL-6 across both correlation methods, while age showed weak or negligible correlations with all laboratory parameters.

Regional Comparison of COVID-19 Diagnostic and Inflammatory Profiles

Table V summarizes the comparison of clinical and laboratory features among COVID-19 patients from three major cities in Punjab. No statistically significant differences were observed across the cities in symptom status, test positivity (PCR, RAT, IgG), or biomarker levels (LDH, D-dimer, CRP, IL-6).

Comparison of SARS-CoV-2 Positivity and Biomarker Levels Between Healthcare Workers and the General Population

Healthcare workers had significantly higher PCR positivity (68.0% vs. 62.6%; $p = 0.041$) and elevated LDH, CRP, and IL-6 levels (all $p < 0.001$) compared to

Table V: Distribution of diagnostic test positivity and inflammatory markers among individuals screened for COVID-19 in three major cities of Punjab, Pakistan

City	n	Asymptomatic (n, %)	Symptomatic (n, %)	PCR+ (n, %)	RAT+ (n, %)	IgG Positive (n, %)	Median LDH	Median D-Dimer	Median CRP	Median IL-6
Lahore	471	222 (47.1%)	249 (52.9%)	316 (67.1%)	232 (49.3%)	340 (72.1%)	252	0.4	6.35	36.9
Faisalabad	538	277 (51.5%)	261 (48.5%)	337 (62.6%)	248 (46.1%)	388 (72.2%)	223	0.5	11.4	23.3
Sargodha	491	235 (47.9%)	256 (52.1%)	339 (69.0%)	237 (48.3%)	340 (69.2%)	254	0.5	8.3	36.7
p-value	–	0.3706*	0.3706*	1.0*	1.0*	1.0*	0.7166†	0.5731†	0.5731†	0.7166†

* *p*-values calculated using the Chi-square test for categorical variables

† *p*-values calculated using the Kruskal–Wallis test for non-normally distributed continuous variables

Table VI: Comparison of Symptom Status, PCR Positivity, and Inflammatory Biomarkers between Healthcare Workers and General Population

Variable	Healthcare Workers (n = 984)	General Population (n = 516)	p-value (Test)
Symptomatic (%)	512 (52.0%)	254 (49.2%)	0.328 (Chi-square test)
PCR Positive (%)	669 (68.0%)	323 (62.6%)	0.041 (Chi-square test)
Median LDH (U/L)	254	219	<0.001* (Mann–Whitney U test)
Median D-Dimer (µg/mL)	0.4	0.4	1.000 (Mann–Whitney U test)
Median CRP (mg/L)	9.1	7.45	<0.001* (Mann–Whitney U test)
Median IL-6 (pg/mL)	36.25	27.85	<0.001* (Mann–Whitney U test)

the general population. Symptom prevalence was similar (52.0% vs. 49.2%; $p = 0.328$), and no difference was observed in D-dimer levels ($p = 1.000$).

(Table VI)

Evaluation of RAT Sensitivity and Specificity

RAT showed high sensitivity (97.3%) and specificity (100%) in symptomatic individuals, with PPV and NPV of 100% and 75.6%, respectively. In asymptomatic cases, sensitivity dropped to 10.2%, while specificity and PPV remained at 100%. (Table VII)

Table VII: Performance of the Rapid Antigen Test (RAT) in Asymptomatic and Symptomatic Individuals

RAT Metric	Asymptomatic (%)	Symptomatic (%)	Total (%)
Sensitivity	10.2	97.3	72.3
Specificity	100.0	100.0	100.0
Positive Predictive Value (PPV)	100.0	100.0	100.0
Negative Predictive Value (NPV)	63.7	75.6	64.9

DISCUSSION

This analysis provides an overview of demographic, diagnostic, and inflammatory markers in symptomatic SARS-CoV-2 infection within a large Punjab cohort. Symptomatic subjects exhibited higher viral detection, antibody prevalence, and inflammatory markers than asymptomatic individuals. Participant characteristics revealed a significant association between gender and symptomatic status ($p < 0.0001$), with males comprising 63.8% of symptomatic versus 45.9% asymptomatic cases, aligning with global COVID-19 severity disparities⁹. This likely reflects inherent

immunological advantages in females, including X-chromosome-mediated immune gene expression, estrogen-driven antiviral responses, and reduced cardiometabolic comorbidities⁹.

Among 1,500 participants, 66.1% tested PCR-positive, with significantly higher positivity in symptomatic (92.3%) versus asymptomatic individuals (38.8%; $X^2 = 476.08$, $p < 0.0001$). This aligns with evidence of prolonged viral shedding in symptomatic cases, though asymptomatic cases remain transmissible^{10,11}.

The rapid antigen test (RAT) shows a markedly higher positivity rate in symptomatic individuals (89.8%) than in asymptomatic (4.0%) groups in this study. These findings are consistent with prior studies. Symptomatic patients tend to have a higher viral load, especially in the early phases of the disease when symptoms appear. This elevated viral load increases the likelihood of RAT, which targets viral proteins; this is consistent with multiple studies reporting RAT sensitivity up to 90% in symptomatic subjects^{12,13}. RATs are less sensitive than RT-PCR tests, particularly in populations with low viral loads, such as asymptomatic cases. Even among asymptomatic individuals, RAT sensitivity varies by timing and viral load; for example, the RAT shows sensitivity of ~80–90% sensitivity in presymptomatic/early asymptomatic phases but much lower in other phases¹⁴. However, the lower sensitivity in asymptomatic individuals requires cautious interpretation and may warrant confirmatory RT-PCR testing, particularly for negative RAT results in asymptomatic contacts or in screening programs¹⁵.

The significantly higher frequency of IgG antibodies in symptomatic participants (82.2%) compared to asymptomatic ones (59.7%) suggests a more robust

or detectable adaptive immune response following clinically apparent infection. Most symptomatic patients develop strong neutralizing and anti-SARS-CoV-2 IgG antibody responses, often with higher titers than asymptomatic or mild cases. For example, pneumonia patients showed 100% neutralizing antibody production, mild symptoms in about 93.9%, but only about 80% in asymptomatic groups, with lower antibody titers in asymptomatic cases¹⁶. Some studies found that asymptomatic patients have a faster but sometimes less durable spike-directed IgG response than mildly symptomatic cases^{17,18}. Longitudinal studies show antibody responses, including IgG, persist for months in both groups but are usually stronger and sustained longer in symptomatic patients¹⁹. Some population studies found exceptions with higher antibody positivity in asymptomatic individuals in certain age groups, reflecting heterogeneity in immune responses across different demographics and severities²⁰. These findings emphasize that symptomatic COVID-19 cases tend to trigger a stronger, more easily detectable humoral immune response, whereas asymptomatic infections induce a variable, generally lower-magnitude IgG response, yet still contribute to immunity and epidemiological dynamics.

The study data show a statistically significant elevation of inflammatory and coagulation biomarkers (LDH, CRP, D-dimer, and IL-6) in symptomatic COVID-19 patients compared to asymptomatic patients. This aligns well with extensive evidence in the literature on COVID-19 pathophysiology and severity markers. Elevated LDH levels correlate with tissue damage and are commonly higher in symptomatic and severe COVID-19 cases, reflecting cellular injury in the lungs and other organs. Higher median LDH in symptomatic (roughly double that of the asymptomatic individuals) is consistent with disease-related tissue damage seen in the literature^{21,22}. CRP is a widely recognized acute-phase inflammatory marker strongly associated with COVID-19 severity. The observed apparent differences (29 mg/L vs. 0.81 mg/L) in this study are typical of this inflammatory response gradient. Multiple studies report substantially elevated CRP levels in symptomatic and severe cases compared with asymptomatic or mild infections^{21,23,24}. D-dimer indicates coagulation activation and fibrinolysis; elevated levels predict worse prognosis and more severe disease. Studies show significantly higher D-dimer levels in symptomatic and hospitalized patients than in asymptomatic individuals, reflecting COVID-19-associated coagulopathy and increased thrombosis risk^{21,22}. IL-6 is a central cytokine in the COVID-19 cytokine storm and correlates with poor outcomes. Median levels, as seen in our study (i.e., 50 pg/mL in symptomatic versus 5 pg/mL in asymptomatic), mirror this pronounced inflammatory activation. It is frequently reported as markedly elevated in symptomatic and severe patients versus asymptomatic ones^{21,22,24}. Collectively, these findings

highlight distinct inflammatory and prothrombotic profiles between symptomatic and asymptomatic groups, substantiating the role of these biomarkers in predicting disease progression and informing clinical management.

The comparative analysis of COVID-19 clinical and laboratory features among patients from Lahore, Faisalabad, and Sargodha revealed no statistically significant differences in key parameters, including the prevalence of symptoms, RT-PCR positivity, RAT positivity, and IgG seropositivity. This uniformity suggests a broadly consistent epidemiological and immunological pattern of COVID-19 infection across these major cities of Punjab. Similarly, inflammatory and biochemical biomarkers, including LDH, D-dimer, CRP, and IL-6, did not show significant variation among these regions. These findings imply comparable disease processes and immune-inflammatory responses regardless of geographic location within the province. For inflammatory and coagulation biomarkers such as LDH, CRP, D-dimer, and IL-6, systematic reviews and cohort studies report these markers are consistently elevated in symptomatic and severe COVID-19 patients²⁵⁻²⁷. The similarity in symptom proportions and PCR/RAT positivity across cities in Punjab aligns with the finding that geographic differences may be secondary to other patient-level factors^{25,27}.

Our comparative analysis revealed that healthcare workers had a significantly higher SARS-CoV-2 PCR positivity rate than the general population, reinforcing their classification as a high-risk group for COVID-19 infection due to occupational exposure^{28,29}. Despite similar proportions of symptomatic individuals in both groups, healthcare workers exhibited significantly elevated levels of inflammatory biomarkers LDH, CRP, and IL-6, suggesting a more pronounced systemic inflammatory response^{30,31}. These findings may be attributable to greater viral exposure among healthcare workers, potentially resulting in higher viral loads or repeated antigenic stimulation that amplifies immune activation^{32,33}. Our results align with existing literature underscoring the vulnerability of healthcare workers to SARS-CoV-2 infection and highlight the need for continued protective measures and immunological monitoring within this key occupational population²⁸⁻³⁰.

CONCLUSION

This extensive cohort study from Punjab, Pakistan, provides critical insights and offers valuable lessons for future respiratory pandemics. Key findings demonstrate that rapid antigen tests (RATs) exhibit markedly high positivity (89.8%) and utility in symptomatic individuals. This underscores the primary role of RATs in early symptomatic case detection while highlighting the necessity for confirmatory PCR testing in asymptomatic screening contexts due to the risk of false negatives. Furthermore, symptomatic infection was characterized by a significantly more

robust humoral immune response and a pronounced elevation of key inflammatory and coagulation biomarkers (LDH, CRP, D-dimer, IL-6) compared with asymptomatic infection. Collectively, these findings advocate for tailored public health strategies in future pandemics: prioritizing RATs for symptomatic individuals while recognizing their limitations for controlling asymptomatic spread, using inflammatory markers for prognosis, and implementing enhanced protective measures for high-risk occupational groups such as healthcare workers.

Acknowledgements: The authors gratefully acknowledge Dr. Mohsin Khurshid for his valuable suggestions and critical review of the manuscript

Ethical Permission: Government College University, Faisalabad, Pakistan, ERC letter No.GCUF/ERC/10-A.

Disclaimer: This manuscript is part of a thesis project.

Conflict of interest: There is no conflict of interest between the authors.

Financial Disclosure / Grant Approval: No funding agency was involved in this research.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Mustafa AU: Conceptualization, methodology, investigation, data curation, formal analysis, writing original draft.

Rasool MH: Conceptualization, supervision, validation, interpretation, writing, review and editing.

Aslam B: Methodology, resources, critical revision of the manuscript, writing, review and editing.

Shafique M: Data curation, formal analysis, visualization, writing, review and editing.

All authors read and approved the final version of the manuscript.

REFERENCES

1. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med*. 2020; 26 (5): 672-5.
2. Ozawa T, Chubachi S, Namkoong H, Nemoto S, Ikegami R, Asakura T et al. Predicting coronavirus disease 2019 severity using explainable artificial intelligence techniques. *Scientif Rpts*. 2025; 15 (1): 9459.
3. Dinnes J, Sharma P, Berhane S, van Wyk SS, Nyaaba N, Domen J et al. Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database System Rev*. 2022; 7(7): Cd013705.
4. Fernandes RS, de Oliveira Silva J, Gomes KB, Azevedo RB, Townsend DM, de Paula Sabino A et al. Recent advances in point of care testing for COVID-19 detection. *Biomed Pharmacother*. 2022; 153: 113538.
5. Schuit E, Veldhuijzen IK, Venekamp RP, van den Bijllaardt W, Pas SD, Lodder EB et al. Diagnostic accuracy of rapid antigen tests in asymptomatic and presymptomatic close contacts of individuals with confirmed SARS-CoV-2 infection: cross-sectional study. *BMJ (Clin Res ed)*. 2021; 374: n1676.
6. Statsenko Y, Al Zahmi F, Habuza T, Gorkom KN, Zaki N. Prediction of COVID-19 severity using laboratory findings on admission: informative values, thresholds, ML model performance. *BMJ Open*. 2021; 11(2): e044500.
7. Saricaoglu EM, Coskun B, Ayhan M, Akinci E, Kayaaslan B, Aypak A et al. A New Laboratory Tool for COVID-19 Severity Prediction, CENIL Score. *Diagnostics (Basel, Switzerland)*. 2024; 14 (22):2557..
8. Zeng H, Ma Y, Zhou Z, Liu W, Huang P, Jiang M, et al. Spectrum and Clinical Characteristics of Symptomatic and Asymptomatic Coronavirus Disease 2019 (COVID-19) With and Without Pneumonia. *Front Med*. 2021; 8: 645651.
9. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*. 2020; 588(7837): 315-20.
10. Wu J, Liu X, Zhou D, Qiu G, Dai M, Yang Q et al. Identification of RT-PCR-Negative Asymptomatic COVID-19 Patients via Serological Testing. *Front Public Health*. 2020; 8: 267.
11. Al-Rifai RH, Acuna J, Al Hossany FI, Aden B, Al Memari SA, Al Mazrouei SK et al. Epidemiological characterization of symptomatic and asymptomatic COVID-19 cases and positivity in subsequent RT-PCR tests in the United Arab Emirates. *PloS One*. 2021; 16(2): e0246903.
12. Badawy ER, Ezz El-Din AM, El Zohne RA. Evaluation of diagnostic performance of a rapid antigen test in diagnosing COVID-19. *Egypt J Immunol*. 2023; 30(1): 14-9.
13. Wertenauer C, Dressel A, Wieland E, Wertenauer HJ, Braitmaier H, Straub A et al. Diagnostic performance of rapid antigen testing for SARS-CoV-2: the COVID-19 AntiGen (COVAG) extension study. *Front Med*. 2024; 11: 1352633.
14. Winkel B, Schram E, Gremmels H, Debast S, Schuurman R, Wensing A, et al. Screening for SARS-CoV-2 infection in asymptomatic individuals using the Panbio COVID-19 antigen rapid test (Abbott) compared with RT-PCR: a prospective cohort study. *BMJ Open*. 2021; 11 (10): e048206.
15. Pray IW, Ford L, Cole D, Lee C, Bigouette JP, Abedi GR et al. Performance of an Antigen-Based Test for Asymptomatic and Symptomatic SARS-CoV-2 Testing at Two University Campuses - Wisconsin, September-October 2020. *MMWR Morb Mortal Wkly Rep*. 2021; 69(5152): 1642-7.

16. Ko JH, Joo EJ, Park SJ, Baek JY, Kim WD, Jee J et al. Neutralizing Antibody Production in Asymptomatic and Mild COVID-19 Patients, in Comparison with Pneumonic COVID-19 Patients. *J Clin Med*. 2020; 9(7):2268.
17. Serwanga J, Ankunda V, Sembera J, Kato L, Oluka GK, Baine C et al. Rapid, early, and potent Spike-directed IgG, IgM, and IgA distinguish asymptomatic from mildly symptomatic COVID-19 in Uganda, with IgG persisting for 28 months. *Front Immunol*. 2023; 14: 1152522.
18. Ebrahimpur M, Hajilooi M, Solgi G, Rastegari-Pouyani M. Serum Levels of IL-21 and IL-27 Do not Reflect differential Avidity of Anti-SARS-CoV-2 IgG Antibodies in Symptomatic and Asymptomatic COVID-19 Patients. *Iran J Allergy Asthma Immunol*. 2025; 24(3): 396-402.
19. Efrati S, Catalogna M, Abu Hamed R, Hadanny A, Bar-Chaim A, Benveniste-Levkovitz P et al. Early and long-term antibody kinetics of asymptomatic and mild disease COVID-19 patients. *Scientif Rpts*. 2021; 11(1): 13780.
20. Kumar D, Burma A, Mandal AK, Joshy V. A Comparative Analysis of COVID-19 IgG Antibody Level and Socio-Demographic Status in Symptomatic and Asymptomatic Population of South Andaman, India. *Cureus*. 2022; 14(2): e22398.
21. Mahdi DS, Al-Shawk RS, Hamid ZA, Abed SM. Comparison Study of the Inflammatory Biomarkers and Cytokine Levels in COVID-19 Patients. *Mustansiriya Med J*. 2024; 23(1): 7-11.
22. Chaudhary R, Garg J, Houghton DE, Murad MH, Kondur A, Chaudhary R, et al. Thromboinflammatory Biomarkers in COVID-19: Systematic Review and Meta-analysis of 17,052 Patients. *Mayo Clinic proceedings Innovations, Quality & Outcomes*. 2021; 5(2): 388-402.
23. Paranga TG, Pavel-Tanasa M, Constantinescu D, Plesca CE, Petrovici C, Miftode IL et al. Comparison of C-reactive protein with distinct hyperinflammatory biomarkers in association with COVID-19 severity, mortality and SARS-CoV-2 variants. *Front Immunol*. 2023; 14: 1213246.
24. Lampart M, Zellweger N, Bassetti S, Tschudin-Sutter S, Rentsch KM, Siegemund M, et al. Clinical utility of inflammatory biomarkers in COVID-19 in direct comparison to other respiratory infections: A prospective cohort study. *PloS One*. 2022; 17(5): e0269005.
25. Semiz S. COVID-19 biomarkers: What did we learn from systematic reviews? *Front Cell Infect Microbiol*. 2022; 12: 1038908.
26. Weidmann MD, Ofori K, Rai AJ. Laboratory Biomarkers in the Management of Patients With COVID-19. *Am J Clin Pathol*. 2021; 155(3): 333-42.
27. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19: A systematic review. *Life Sci*. 2020; 254: 117788.
28. Nguyen LH, Drew DA, Graham MS, Joshi AD, Guo CG, Ma W et al. Risk of COVID-19 among frontline healthcare workers and the general community: a prospective cohort study. *Lancet Public Health*. 2020; 5(9): e475-e83.
29. Lai X, Wang M, Qin C, Tan L, Ran L, Chen D et al. Coronavirus Disease 2019 (COVID-2019) Infection Among Health Care Workers and Implications for Prevention Measures in a Tertiary Hospital in Wuhan, China. *JAMA Network Open*. 2020; 3(5): e209666.
30. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. 2020; 130(5): 2620-9.
31. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet (Lond, Engl)*. 2020; 395(10223): 497-506.
32. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet (Lond Engl)*. 2020; 395(10229): 1054-62.
33. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. 2020; 323(11): 1061-9.

